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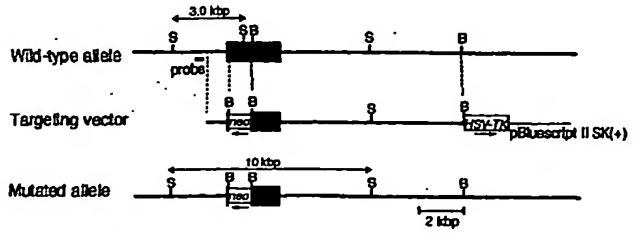
(54) **RECEPTOR PROTEIN SPECIFICALLY RECOGNIZING BACTERIAL DNA**

(57) The present invention provides a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence, a genomic DNA encoding it, an experimental animal model useful for examining responsiveness of a host immune cell against a bacterial infectious disease. DNA encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is screened by BLAST search, a number of EST clones having high homology with various TLRs is screened, these clones are used as a probe to isolate a full-length cDNA from mouse macrophage cDNA library, and the sequence of bases of the cDNA is analyzed to confirm that it is TLR9 comprising a conserved regions such as LRR and TIR regions, and then a knockout mouse is produced to confirm that TLR9 is a receptor protein of oligonucleotides having an unmethylated CpG sequence of bacterial DNA.

FIG. 1



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in the TLR family such as LRR and TIR domains are present. We generated TLR9 knockout mice, showed that TLR9 is a receptor protein to the oligonucleotides comprising an unmethylated CpG sequence of bacterial DNA and completed the invention.

DISCLOSURE OF THE INVENTION

[0010] The present invention relates to DNA encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence (claim 1), the protein according to claim 1 wherein a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is either of the following proteins (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No: 2, or (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No: 2, and having reactivity against bacterial DNA having an unmethylated CpG sequence (claim 2), the DNA according to claim 1 comprising the sequence of bases shown in Seq. ID No: 1 or its complementary sequence, or part or whole of the sequences (claim 3), the DNA according to claim 1 which hybridizes with the DNA comprising a gene according to claim 3 under a stringent condition (claim 4), the protein according to claim 1 wherein a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is either of the following proteins (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No: 4, or (b) a protein comprising a sequence of amino acids wherein one or more of amino acid are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No: 4, and having reactivity against bacterial DNA having an unmethylated CpG sequence (claim 5), the DNA according to claim 1 comprising the sequence of bases shown in Seq. ID No: 3 or its complementary sequence, or part or whole of the sequences (claim 6), and the DNA according to claim 1 which hybridizes with the DNA comprising the gene according to claim 6 under a stringent condition (claim7).

[0011] The present invention also relates to a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence (claim 8), the protein according to claim 8 comprising the sequence of amino acids shown in Seq. ID No: 2 (claim 9), the protein according to claim 8 comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted or added in the sequence of amino acids shown in Seq. ID No: 2 (claim 10), the protein according to claim 8 comprising the sequence of amino acids shown in Seq. ID No: 4 (claim 11), and the protein according to claim 8 comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted or added in the sequence of amino acids shown in Seq. ID No: 4 (claim 12).

[0012] The present invention also relates to a fusion protein comprising the protein according to any one of claims 8 to 12 fused with a marker protein and/or a peptide tag (claim 13), an antibody specifically bound to the protein according to any one of claims 8 to 12 (claim 14), the antibody according to claim 14 which is a monoclonal antibody (claim 15), a host cell comprising an expression system expressing the protein according to any one of claims 8 to 12 (claim 16).

[0013] The present invention also relates to a non-human animal wherein a gene encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is excessively expressed (claim 17), a non-human animal wherein a gene function encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is destroyed on a chromosome (claim 18), the non-human animal according to claim 18 having no reactivity against bacterial DNA having an unmethylated CpG sequence (claim 19), the non-human animal according to any one of claims 17 to 19 characterized in that a rodent animal is a mouse (claim 20).

[0014] The present invention also relates to a method of preparing a cell expressing a protein having reactivity against bacterial DNA having an unmethylated CpG sequence characterized in that the DNA according to any one of claims 1 to 7 is introduced into a cell wherein a gene function encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is destroyed on a chromosome (claim 21), and a cell expressing a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence obtained by the method of preparing a cell expressing a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence according to claim 21 (claim 22).

[0015] The present invention also relates to screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising steps of: in vitro culturing a cell expressing a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence in the presence of a target substance, and measuring/evaluating TLR9 activity (claim 23), a screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising steps of: administrating a target substance to a non-human animal wherein a gene function encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is destroyed on a chromosome, and measuring/evaluating TLR9 activity of macrophages or spleen cells obtained from the non-human animal (claim 24), a screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising steps of: administrating a target substance to a non-human animal wherein a gene encoding a receptor protein specifically recognizing bacterial DNA having an

sequence such as the one shown in Seq. ID No: 1, DNA comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted or added in a sequence of amino acids shown in Seq. ID No: 2, and which can specifically recognize bacterial DNA having the unmethylated CpG sequence mentioned above, or DNA hybridized with the DNA under stringent conditions and encoding a protein that can specifically recognize bacterial DNA having the unmethylated CpG sequence mentioned above. These can be prepared by well known methods based on the information of DNA sequence such as mouse RAW264.7 cDNA library or 129/SvJ mouse gene library for mouse-derived TLR9.

[0021] Further, it is possible to obtain DNA encoding a receptor protein specifically recognizing bacterial DNA having an immune-inducing unmethylated CpG sequence which has the same effect as TLR9, a receptor protein, by hybridizing mouse-derived DNA library with part or whole of a sequence of bases shown in Seq. ID No: 1 or its complementary sequence under stringent conditions to isolate the DNA hybridized with the probe. Conditions on hybridization to obtain the DNA can, for example, be hybridization at 42°C and wash treatment at 42°C with a buffer containing 1% × SSC and 0.1% of SDS, and more preferably be hybridization at 65°C and wash treatment at 65°C with a buffer containing 0.1 × SSC and 0.1% of SDS. Furthermore, beside the temperature conditions mentioned above, there are various factors effecting the stringency of hybridization, and it is possible for a person skilled in the art to realize the stringency equivalent to the stringency of hybridization illustrated above.

[0022] A fusion protein in the present invention can be the one obtained by combining a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence derived from mouse, human, and others with a marker protein and/or a peptide tag. A marker protein can be any marker protein previously well known, and can be exemplified by alkaline phosphatase, Fc region of an antibody, HRP, GFP and others. As a peptide tag in the present invention, it can be concretely exemplified by previously well-known peptide tags such as Myc tag, His tag, FLAG tag, GST tag. The fusion protein can be produced by a normal method, and is useful in purifying a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence by using affinity of Ni-NTA and His tag, detecting a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence, measuring of the amount of antibodies against a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence and as a research reagent in other relevant fields.

[0023] As an antibody specifically bound to a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence in the present invention, it can be concretely exemplified by immune-specific antibodies such as a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a single-chain antibody, a humanized antibody. These antibodies can be produced by a normal method by using a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence mentioned above as an antigen, and a monoclonal antibody is preferable in its specificity among them. The antibody specifically bound to a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence such as a monoclonal antibody and others is useful, for example, in diagnosing diseases caused by the mutation or deletion of TLR9 or elucidating the molecular mechanism controlling TLR9.

[0024] An antibody against a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence can be produced by administrating a fragment containing a receptor protein or an epitope specifically recognizing bacterial DNA having the unmethylated CpG sequence in animals (preferably, non-human), or a cell expressing the protein on the surface of its membrane by a conventional protocol, and any method can be used such as hybridoma method (Nature 256, 495-497, 1975), trioma method, human B cell hybridoma method (Immunology Today 4, 72, 1983), and EBV-hybridoma method (MONOCLONAL ANTIBODIES AND CANCER THERAPY, 77-96, Alan R. Liss, Inc., 1985), which are used for preparing monoclonal antibodies and brings an antibody produced by the cultured successive cell lines. The following explains a method of producing a monoclonal antibody specifically bound to mouse-driven TLR9, that is, an mTLR9 monoclonal antibody, with mouse-driven TLR9 as an example of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence.

[0025] The mTLR9 monoclonal antibody can be produced by a normal method of culturing hybridoma producing mTLR9 monoclonal antibody in vivo or in vitro. For example, in an in vivo systems they can be obtained by culturing in the visceral cavity of rodents, preferably of mice or rats, and in an in vitro system they can be obtained by culturing in a medium for culturing animal cells. A medium used for culturing hybridoma in an in vitro system can be exemplified by cell culture media such as RPMI1640 or MEN and others comprising antibiotics such as streptomycin or penicillin.

[0026] The hybridoma producing mTLR9 monoclonal antibody can be produced by immunizing BALB/c mouse with TLR9, a receptor protein obtained from mouse and others, fusing a spleen cell from an immunized mouse and a mouse NS-1 cell (ATCC TIB-18) by a normal method, and screening them by immunofluorescence staining patterns. A method of separating/isolating the monoclonal antibody can be any one as long as it is a method usually used for purifying proteins, and liquid chromatography such as affinity chromatography and others can be a concrete example.

[0027] It is also possible to apply the method of a single-chain antibody (US Patent No. 4946778) to produce single-chain antibodies against receptor proteins specifically recognizing bacterial DNA having the above-mentioned unmethylated CpG sequence of the present invention. Further, it is possible to use transgenic mice or other mammals and

means that the reactivity against stimuli by bacterial DNA shown by an organism, or a cell, a tissue or an organ constituting the organism is declined or almost totally lost. Therefore, a non-human animal with refractory against bacterial DNA having an unmethylated CpG sequence in the present invention is a non-human animal such as mice, rats, or rabbits, wherein the an organism's reactivity against bacterial DNA, or a cell, a tissue or an organ constituting the organism is declined or almost totally lost. Further, stimuli by bacterial DNA can be exemplified by an in vivo stimulus caused by administrating bacterial DNA to an organism, or an in vitro stimulus caused by contacting cells separated from an organism with bacterial DNA. Concretely, a non-human animal such as TLR9 knockout mice wherein TLR9 gene functions are destroyed on the chromosome can be an example.

[0034] A homozygote non-human animals born following Mendel's Law includes mice deficient of or excessively expressing receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence and their wild-type littermates, and it is preferable to use wild-type non-human animals, that is, the same kind of animal as a non-human animal wherein gene functions encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence are destroyed or are excessive, more preferably their littermate animals, for example, during the screening of the present invention described below because accurate comparative experiments can be carried out at the level of individuals by using the homozygote non-human animals with its receptor proteins destroyed or the one with receptor proteins expressing excessively or the wild-type non-human animals born from the same mother at the same time. In the following, a method of producing non-human animals wherein gene functions encoding a receptor protein specifically recognizing bacterial DNA having the unmethylated CpG sequence are destroyed or excessively expressed on the chromosome is explained using knockout mice or transgenic mice whose receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence as an example.

[0035] For example, as for a mouse wherein gene functions encoding receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence are destroyed on a chromosome such as TLR9, that is, a knockout mouse lacking receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence, gene fragments obtained from mouse gene library by a method of PCR or the like are used to screen genes encoding receptor proteins specifically recognizing bacterial DNA having the unmethylated CpG sequence, subclone a gene encoding a receptor protein specifically recognizing bacterial DNA having the screened unmethylated CpG sequence with viral vectors and others, and specified by DNA sequencing. Whole or part of the gene in the clone encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is substituted with pMC1 neo gene cassette and others, and a targeting vector is produced by introducing diphtheria toxin A fragments (DT-A) genes or herpes simplex virus thymidine kinase (HSV-tk) genes and others on 3'-end side.

[0036] The produced targeting vector is linearlized, introduced into ES cells by electroporation method and others, homologous recombination is performed, and ES cells which has caused homologous recombination by antibiotics such as G418 or gancyclovir (GANC) and others are selected from the homologous recombinants. It is preferable to confirm by Southern blot technique that the selected ES cells are targeted recombinants. The clones of the confirmed ES cells are introduced to mouse blastocysts by microinjection, and the blastcysts are returned to recipient mice, and chimera mice were produced. The chimera mouse was intercrossed with a wild-type mouse to produce a heterozygote mouse, and the heterozygote mice are intercrossed to produce a knockout mouse lacking a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence in the present invention. Further, a method of confirming whether knockout mice lacking a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is obtained, for example, may be examined by Northern blot technique, which isolates RNA from the mouse obtained by the method mentioned above, or the expression in the mice may be examined by Western blot technique.

[0037] The fact that the produced TLR9 knockout mouse is refractory against bacterial DNA having an unmethylated CpG sequence can be confirmed by measuring the levels of the production of TNF-α, IL-6, IL-12, IFN-γ and others in the cells whose CpG ODN was contacted in vivo or in vitro with immune cells such as macrophages, mononuclear cells, dendritic cells from TLR9 knockout mice, the proliferation of response of spleen B cells, the expression of antibodies such as CD40, CD80, CD86, MHC class II on the surface of spleen B cells, and the activation of molecules on the signal transduction pathway of NF-κ B, JNK, IRAK and others. The knockout mice lacking TLR9 in the present invention can be used to elucidate functional mechanisms of bacterial DNA and others having an unmethylated CpG sequence and to developing vaccine against bacterial infections.

[0038] Transgenic mice lacking receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence can be generated by constructing introduced genes by fusing chicken β actin, mouse neurofilament, promotors such as SV40, and rabbit β -globin, polyA such as SV40 or intron with cDNA encoding receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence such as TLR9, microinjecting the introduced genes to pronucleus of mouse fertilized eggs, transplanting the obtained cells to an oviduct of recipient mice after culturing them, then breeding the transplanted animals, and selecting child mice having the cDNA from born child mice. Further, selection of the child mice having cDNA can be performed by dot hybridization wherein crude cDNA was extracted from mouse tails and others, and genes encoding receptor proteins specifically recognizing bacterial DNA

of spleen cell activities shown by the spleen cells, a method comprising the steps of making macrophages or spleen cells obtained from non-human animals whose gene functions encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence contact in vitro with bacterial DNA having an unmethylated CpG sequence, then culturing the macrophages or spleen cells in the presence of target substances, and measuring/evaluating the levels of macrophage activities shown by the macrophages or the levels of spleen cell activities shown by the spleen cells, and a method of comprising the steps of first administrating target substances to non-human animals whose gene functions encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence on a chromosome first, then culturing the macrophages or spleen cells obtained from the non-human animals in the presence of bacterial DNA having an unmethylated CpG sequence, and measuring/evaluating the levels of macrophage activities shown by the macrophages or the levels of spleen cell activities shown by the spleen cells, a method comprising the steps of first administrating target substances to non-human animals whose gene functions encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence is destroyed on a chromosome, then infecting the non-human animals by bacteria, and measuring/evaluating the levels of macrophage activities shown by macrophages or the levels of spleen cell activities shown by the spleen cells obtained from non-human animals, a method of the steps of first administrating target substance to non-human animals whose gene functions encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence on a chromosome, and measuring/evaluating the levels of macrophage activities shown by macrophages or the levels of spleen cell activities shown by spleen cells obtained from the non-human animals, a method comprising the steps of first infecting with bacteria non-human animals whose gene functions encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence are destroyed on a chromosome, then culturing macrophages or spleen cells obtained from the non-human animals in the presence of target substances, and measuring/evaluating the levels of macrophage activities shown by macrophages or the levels of spleen cell activities shown by spleen cells obtained from the non-human animals, a method comprising the steps of administrating target substances to non-human animals whose gene functions are encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence are destroyed, infecting the non-human animals by bacteria, and measuring/evaluating the levels of macrophage activities or spleen cell activities in the non-human animals, and a method comprising the steps of infecting non-human animals whose gene functions encoding proteins having reactivity against bacterial DNA having an unmethylated CpG sequence are destroyed on a chromosome first, then administrating the target substances to the non-human animals, and measuring/evaluating the levels of macrophage activities or spleen cell activities in the non-human animals. Although as bacterial DNA having an unmethylated CpG sequence used in the screening methods, it is preferable to use CpG ODN (TCC-ATG-ACG-TTC-CTG-ATG-CT: Seq. ID No: 5), it is not limited to this.

[0045] The present invention also relates to a kit used to diagnose diseases relating to the activity or expression of receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence by comparing a sequence of DNA encoding receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence in a test body with a sequence of DNA encoding receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence in the present invention. The detection of mutated DNA encoding receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence can be carried out by detecting genetically mutated individuals at the level of DNA, and is effective for diagnosing diseases caused by hypotypic expression, hypertypic expression or mutated expression of receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence. Although a test body used in the detection can concretely be exemplified by genomic DNA of cells from subjects obtainable by biopsy from blood, urine, saliva, tissue and others, RNA, or cDNA, it is not limited to these. In using the test body, it is possible to use the ones amplified by PCR and others. The deficiency or insertional mutation in sequences of bases can be detected by the changes of amplified products in size compared with normal genes, and point mutation can be identified by hybridizing the amplified DNA with the gene encoding receptor proteins specifically recognizing bacterial DNA having labeled unmethylated CpG sequence. It is possible to diagnose or conclude diseases relevant to activity or expression of receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence by detecting mutation of a gene encoding receptor proteins specifically recognizing bacterial DNA having an unmethylated CpG sequence.

[0046] The present invention also relates to a probe diagnosing a disease related to activities or expressions of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising whole or part of antisense chain of DNA or RNA encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence, and a kit used to diagnose diseases relating to activities or expressions of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising an antibody specifically bound to a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence of the probe and/or in the present invention. A probe used for the diagnosis is whole or part of an antisense chain of DNA (cDNA) or RNA (cRNA) encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence, and there is no limitations on the probe as long as it is long enough (at least 20 bases or more) to establish as a probe. In order to make an antibody specifically bound to a receptor protein specifically recognizing

compositions were found.

Example 3: Preparation of peritoneal macrophages

[0054] 2ml of 4% thioglycolic acid medium (DIFCO) was injected to each peritoneum of wild-type mice and TLR9 knockout mice ($TLR9^{-/-}$), peritoneal exudation cells were isolated from peritonea from each mouse after 3 days, the cells were cultured in RPMI1640 medium to which 10% of fetal bovine serum (GIBCO) was added at 37°C for 2 hours, and remove the unattached cells by washing with ice-chilled Hank's buffered salt solution (HBSS; GIBCO), and the attached cells were used as peritoneal macrophages in the following experiments.

Experiment 4: Response to bacterial DNA having an unmethylated CpG sequence in TLR9 knockout mice

[0055] It has recently been shown that the response of CpG ODN (oligodeoxynucleotide) is dependent on MyD88, an adopter protein in a signaling transduction pathway mediating TLR. Although the MyD88 knockout mice do not show response to CpG ODN, TLR2 knockout mice or TLR4 knockout mice show normal response to it. This shows that CpG ODN recognizes TLRs other than TLR2 and TLR4, and then the response of a TLR9 knockout mouse against CpG ODN was examined. First, the amount of producing inflammatory cytokines in peritoneal macrophages were measured in the following way.

[0056] The macrophages prepared in Example 3 are co-cultured with various concentrations of CpG ODN shown in Fig. 5 (0.1 or 1.0μM; TIB MOLBIOL; TCC-ATG-ACG-TTC-CTG-ATG-CT), PGN (10μg/ml; Sigma and Fluka; derived from Staphylococcus aureus), LPS (1.0 μg/ml; Sigma; derived from Salmonella minnesota Re-595) in the presence or absence of INFγ (30 unit/ml). The concentrations of TNFα, IL-6 and IL-12 p40 in the supernatants after culturing were measured by ELISA, and the results are shown in Fig. 5. The results show that the macrophages from wild-type mice (Wild-type) produce TNFα, IL-6 and IL-12 in response to CpG ODN, and further stimulation by IFNγ and CpG ODN increases the amount of producing TNFα, IL-6 and IL-12. However, the macrophages derived from TLR9 knockout mice ($TLR9^{-/-}$) did not produce a detectable level of inflammatory cytokines in response to CpG ODN even in the presence of IFNγ. Further, it was found that the macrophages derived from wild-type mice and TLR9 knockout mice produce almost the same level of TNFα, IL-6 and IL-12 in response to LPS or PGN (Fig. 5). Each experimental result shows the average level of n=3. N.D. in the figures means not detected.

[0057] Response of spleen cells from wild-type mice (Wild-type) and TLR9 knockout mice ($TLR9^{-/-}$) against CpG ODN or LPS was also examined. The spleen cells from each mouse (1×10^5) were isolated to culture in 96 well plates by CpG DNA or LPS of various concentrations shown in Fig. 6, and the spleen cells were stimulated. 40 hours later from culturing, 1μ Ci of [^{3}H]-timidine (Dupont) was added, and then further cultured for 8 hours. The amount of uptaking [^{3}H]-timidine was measured by β scintillation counter (Packard) (Fig. 6). The results that although the spleen cells from wild-type mice promote cell proliferating reactions depending on the amount of administrating CpG ODN or LPS, the spleen cells from TLR9 knockout mice did not show any cell proliferating reaction by CpG ODN even with the stimulus of any concentration of CpG ODN. Further, the amount of expressing Major Histocompatibility Complex (MHC) class II on the surface of B cells derived from wild-type mice in response to CpG ODN was increased. However, such increase of the amount of expressing MHC class II induced by CpG ODN in B cells derived from TLR9 knockout mice was not observed. These facts show that the macrophages or B cells from TLR9 knockout mice specifically lack the response against CpG ODN.

[0058] Next, it is well known that DNA derived from bacteria comprising CpG ODN potentially stimulates dendritic cells, and supports the development of Th1 cell (EMBO J. 18, 6973-6982, 1999, J. Immunol. 161, 3042-3049, 1998, Proc. Natl. Acad. Sci. USA 96, 9305-9310, 1999). Then, the production of CpG ODN-inducing cytokines and the up-regulation of the surface molecule of dendritic cells derived from bone marrow were examined. The bone marrow cells from wild-type mice (Wild-type) or TLR9 knockout mice ($TLR9^{-/-}$) were cultured with 10ng/ml mouse granulocyte macrophage-colony stimulating factor (Peprotech) in RPMI1640 medium supplemented with 10% fetal bovine serum (J. Exp. Med. 176, 1693-1702, 1992), at day 6 of the culture, immature dendritic cells were harvested and cultured in the presence or absence of 0.1 μM CpG ODN or 0.1 μg/ml LPS in RPMI1640 medium supplemented with 10% fetal bovine serum for 2 days. After the culture, the concentration of IL-12 p40 in the supernatants was measured by ELISA (Fig. 7). The result shows that the dendritic cells derived from wild-type mice produced IL-12 in response to CpG ODN while the dendritic cells derived from TLR9 knockout mice did not induce the production of IL-12 in response to CpG ODN.

[0059] After culturing in RPMI supplemented with 10% fetal bovine serum was cultured which contains 10ng/ml mouse granulocyte macrophage-colony stimulating factor (Peprotech), the dendritic cells harvested at day 6 were stained with biotinylated antibodies against CD40, CD80, CD86 or MHC class II, developed with streptovidine labeled with phycoerythrin (PE; PharMingen). The cells were examined by using a FACSCalibur with CELLQuest software (Becton Dickinson) (Fig. 8). The result shows that stimulation by CpG ODN promotes the expression of CD40, CD80, CD86 and MHC class II on the surface of dendritic cells derived from wild-type mouse while it does not promote the

SEQUENCE LISTING

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Ala Arg Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met Asn Phe Ile
        390                 395                 400

aac cag gcc cag ctc ggc atc ttc agg gcc ttc cct ggc ctg cgc tac   1363
Asn Gln Ala Gln Leu Gly Ile Phe Arg Ala Phe Pro Gly Leu Arg Tyr
    405                 410                 415
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                    600                 605                 610

cat atg tgg gcc gag gga gac ctc tat ctg cac ttc ttc caa ggc ctg   1987
His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe Gln Gly Leu
            615                 620                 625

agc ggt ttg atc tgg ctg gac ttg tcc cag aac cgc ctg cac acc ctc   2035
Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu His Thr Leu
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ctg ccc caa acc ctg cgc aac ctc ccc aag agc cta cag gtg ctg cgt   2083
Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln Val Leu Arg
    645                 650                 655

ctc cgt gac aat tac ctg gcc ttc ttt aag tgg tgg agc ctc cac ttc   2131
Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser Leu His Phe
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ctg ccc aaa ctg gaa gtc ctc gac ctg gca gga aac cag ctg aag gcc   2179
Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Gln Leu Lys Ala
                680                 685                 690

ctg acc aat ggc agc ctg cct gct ggc acc cgg ctc cgg agg ctg gat   2227
Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg Arg Leu Asp
            695                 700                 705

gtc agc tgc aac agc atc agc ttc gtg gcc ccc ggc ttc ttt tcc aag   2275
Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe Phe Ser Lys
        710                 715                 720

gcc aag gag ctg cga gag ctc aac ctt agc gcc aac gcc ctc aag aca   2323
Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala Leu Lys Thr
    725                 730                 735

gtg gac cac tcc tgg ttt ggg ccc ctg gcg agt gcc ctg caa ata cta   2371
Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu Gln Ile Leu
740                 745                 750                 755

gat gta agc gcc aac cct ctg cac tgc gcc tgt ggg gcg gcc ttt atg   2419
Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe Met
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gac ttc ctg ctg gag gtg cag gct gcc gtg ccc ggt ctg ccc agc cgg   2467
Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu Pro Ser Arg
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965 970 975

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980 985 990 995

ccc cac cag ccc agt ggt cag cgc agc ttc tgg gcc cag ctg ggc atg 3139
Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln Leu Gly Met
1000 1005 1010

gcc ctg acc agg gac aac cac cac ttc tat aac cgg aac ttc tgc cag 3187
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1015 1020 1025

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Gly Pro Thr Ala Glu
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tccacactca cctcacctct gc 3257

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<213> Homo sapiens

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Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
35 40 45
Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
50 55 60
Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
65 70 75 80
Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
85 90 95
Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
100 105 110
Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115 120 125
Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser
130 135 140
Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser

```
        515                 520                 525
Ser His Asn Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu
    530                 535                 540
Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly
545                 550                 555                 560
Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr
                565                 570                 575
Leu Arg His Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser
            580                 585                 590
Gln Gln Leu Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
        595                 600                 605
Ala Leu Gly His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe
    610                 615                 620
Gln Gly Leu Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu
625                 630                 635                 640
His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln
                645                 650                 655
Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser
            660                 665                 670
Leu His Phe Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Gln
        675                 680                 685
Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg
    690                 695                 700
Arg Leu Asp Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe
705                 710                 715                 720
Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
                725                 730                 735
Leu Lys Thr Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu
            740                 745                 750
Gln Ile Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala
        755                 760                 765
Ala Phe Met Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu
    770                 775                 780
Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser
785                 790                 795                 800
Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp
                805                 810                 815
Asp Cys Phe Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val
            820                 825                 830
Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
        835                 840                 845
Leu Cys Leu Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp
    850                 855                 860
Glu Asp Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln
865                 870                 875                 880
Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu
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gag ctg aag cct cat ggc ctg gtg gac tgc aat tgg ctg ttc ctg aag   259
Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu Phe Leu Lys
                40                  45                  50

tct gta ccc cgt ttc tct gcg gca gca tcc tgc tcc aac atc acc cgc   307
Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn Ile Thr Arg
            55                  60                  65

ctc tcc ttg atc tcc aac cgt atc cac cac ctg cac aac tcc gac ttc   355
Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn Ser Asp Phe
        70                  75                  80

gtc cac ctg tcc aac ctg cgg cag ctg aac ctc aag tgg aac tgt cca   403
Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp Asn Cys Pro
    85                  90                  95

ccc act ggc ctt agc ccc ttg cac ttc tct tgc cac atg acc att gag   451
Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met Thr Ile Glu
100                 105                 110                 115

ccc aga acc ttc ctg gct atg cgt aca ctg gag gag ctg aac ctg agc   499
Pro Arg Thr Phe Leu Ala Met Arg Thr Leu Glu Glu Leu Asn Leu Ser
                120                 125                 130

tat aat ggt atc acc act gtg ccc cga ctg ccc agc tcc ctg gtg aat   547
Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser Leu Val Asn
            135                 140                 145

ctg agc ctg agc cac acc aac atc ctg gtt cta gat gct aac agc ctc   595
Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala Asn Ser Leu
        150                 155                 160

gcc ggc cta tac agc ctg cgc gtt ctc ttc atg gac ggg aac tgc tac   643
Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly Asn Cys Tyr
    165                 170                 175

tac aag aac ccc tgc aca gga gcg gtg aag gtg acc cca ggc gcc ctc   691
Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro Gly Ala Leu
180                 185                 190                 195

ctg ggc ctg agc aat ctc acc cat ctg tct gtg aag tat aac aac ctc   739
Leu Gly Leu Ser Asn Leu Thr His Leu Ser Val Lys Tyr Asn Asn Leu
                200                 205                 210

aca aag gtg ccc cgc caa ctg ccc ccc agc ctg gag tac ctc ctg gtg   787
Thr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr Leu Leu Val
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aac cag gca cag ctc agc atc ttt ggt acc ttc cga gcc ctt cgc ttt   1363
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    405                 410                 415

gtg gac ttg tca gac aat cgc atc agt ggg cct tca acg ctg tca gaa   1411
Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr Leu Ser Glu
420                 425                 430                 435

gcc acc cct gaa gag gca gat gat gca gag cag gag gag ctg ttg tct   1459
Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu Leu Leu Ser
                440                 445                 450

gcg gat cct cac cca gct cca ctg agc acc cct gct tct aag aac ttc   1507
Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser Lys Asn Phe
            455                 460                 465

atg gac agg tgt aag aac ttc aag ttc acc atg gac ctg tct cgg aac   1555
Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu Ser Arg Asn
        470                 475                 480

aac ctg gtg act atc aag cca gag atg ttt gtc aat ctc tca cgc ctc   1603
Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu Ser Arg Leu
    485                 490                 495

cag tgt ctt agc ctg agc cac aac tcc att gca cag gct gtc aat ggc   1651
Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala Val Asn Gly
500                 505                 510                 515

tct cag ttc ctg ccg ctg act aat ctg cag gtg ctg gac ctg tcc cat   1699
Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp Leu Ser His
                520                 525                 530

aac aaa ctg gac ttg tac cac tgg aaa tcg ttc agt gag cta cca cag   1747
Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu Leu Pro Gln
            535                 540                 545

ttg cag gcc ctg gac ctg agc tac aac agc cag ccc ttt agc atg aag   1795
Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser Met Lys
        550                 555                 560

ggt ata ggc cac aat ttc agt ttt gtg gcc cat ctg tcc atg cta cac   1843
Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser Met Leu His
    565                 570                 575

agc ctt agc ctg gca cac aat gac att cat acc cgt gtg tcc tca cat   1891
Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val Ser Ser His
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gta gac tta ctg ttg gag gtg cag acc aag gtg cct ggc ctg gct aat   2467
Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly Leu Ala Asn
            775                 780                 785

ggt gtg aag tgt ggc agc ccc ggc cag ctg cag ggc cgt agc atc ttc   2515
Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser Ile Phe
        790                 795                 800

gca cag gac ctg cgg ctg tgc ctg gat gag gtc ctc tct tgg gac tgc   2563
Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser Trp Asp Cys
    805                 810                 815

ttt ggc ctt tca ctc ttg gct gtg gcc gtg ggc atg gtg gtg cct ata   2611
Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val Val Pro Ile
820                 825                 830                 835

ctg cac cat ctc tgc ggc tgg gac gtc tgg tac tgt ttt cat ctg tgc   2659
Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe His Leu Cys
                840                 845                 850

ctg gca tgg cta cct ttg ctg gcc cgc agc cga cgc agc gcc caa gct   2707
Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser Ala Gln Ala
            855                 860                 865

ctc ccc tat gat gcc ttc gtg gtg ttc gat aag gca cag agc gca gtt   2755
Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln Ser Ala Val
        870                 875                 880

gcg gac tgg gtg tat aac gag ctg cgg gtg cgg ctg gag gag cgg cgc   2803
Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu Glu Arg Arg
    885                 890                 895

ggt cgc cga gcc cta cgc ttg tgt ctg gag gac cga gat tgg ctg cct   2851
Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp Trp Leu Pro
900                 905                 910                 915

ggc cag acg ctc ttc gag aac ctc tgg gct tcc atc tat ggg agc cgc   2899
Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr Gly Ser Arg
                920                 925                 930

aag act cta ttt gtg ctg gcc cac acg gac cgc gtc agt ggc ctc ctg   2947
Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser Gly Leu Leu
            935                 940                 945

cgc acc agc ttc ctg ctg gct cag cag cgc ctg ttg gaa gac cgc aag   2995
Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys
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 65                  70                  75                  80
Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp
                85                  90                  95
Asn Cys Pro Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met
            100                 105                 110
Thr Ile Glu Pro Arg Thr Phe Leu Ala Met Arg Thr Leu Glu Glu Leu
        115                 120                 125
Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser
    130                 135                 140
Leu Val Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala
145                 150                 155                 160
Asn Ser Leu Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly
                165                 170                 175
Asn Cys Tyr Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro
            180                 185                 190
Gly Ala Leu Leu Gly Leu Ser Asn Leu Thr His Leu Ser Val Lys Tyr
        195                 200                 205
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Leu Leu Val Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu
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Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
                245                 250                 255
Arg Cys Asp His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser
            260                 265                 270
Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly
        275                 280                 285
Leu Val Leu Lys Asp Ser Ser Leu His Thr Leu Asn Ser Ser Trp Phe
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Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu
305                 310                 315                 320
Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu
                325                 330                 335
Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala
            340                 345                 350
Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu
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Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu
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Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met
385                 390                 395                 400
Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala
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Leu Arg Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr
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                805                 810                 815
Trp Asp Cys Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val
            820                 825                 830
Val Pro Ile Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe
        835                 840                 845
His Leu Cys Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser
    850                 855                 860
Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
865                 870                 875                 880
Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu
                885                 890                 895
Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp
            900                 905                 910
Trp Leu Pro Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr
        915                 920                 925
Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
    930                 935                 940
Gly Leu Leu Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945                 950                 955                 960
Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His
                965                 970                 975
Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
            980                 985                 990
Leu Phe Trp Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln
        995                1000                1005
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<220>
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Claims

1. DNA encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence.

20. The non-human animal according to any one of claims 17 to 19 **characterized in that** a rodent animal is a mouse.

21. A method of preparing a cell expressing a protein having reactivity against bacterial DNA having an unmethylated CpG sequence **characterized in that** the DNA according to any one of claims 1 to 7 is introduced into a cell wherein a gene function encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is destroyed on a chromosome.

22. A cell expressing a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence obtained by the method of preparing a cell expressing a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence according to claim 21.

23. A screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising steps of: in vitro culturing a cell expressing a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence in the presence of a target substance, and measuring/evaluating TLR9 activity.

24. A screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising steps of: administrating a target substance to a non-human animal wherein a gene function encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is destroyed on a chromosome, and measuring/evaluating TLR9 activity of macrophages or spleen cells obtained from the non-human animal.

25. A screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising steps of: administrating a target substance to a non-human animal wherein a gene encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence is excessively expressed, and measuring/evaluating TLR9 activity of macrophages or spleen cells obtained from the non-human animal.

26. A screening method for an agonist or an antagonist of a protein having reactivity against bacterial DNA having the unmethylated CpG sequence according to either of claims 24 or 25 using a mouse as a non-human animal.

27. An agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence obtained by the screening method for an agonist or an antagonist of a receptor protein specifically recognizing bacterial DNA having the unmethylated CpG sequence according to any one of claims 23 to 26.

28. A pharmaceutical composition comprising whole or part of a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence as an active component.

29. A pharmaceutical composition comprising the agonist or antagonist according to claim 27 as an active component.

30. A kit used to diagnose diseases related to the deletion, substitution and/or addition in a sequence of DNA encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence comprising the DNA according to claim 3, which can compare a sequence of DNA encoding a receptor protein specifically recognizing bacterial DNA having an unmethylated CpG sequence in a test body with a sequence of bases in the DNA according to claim 3.

FIG. 4

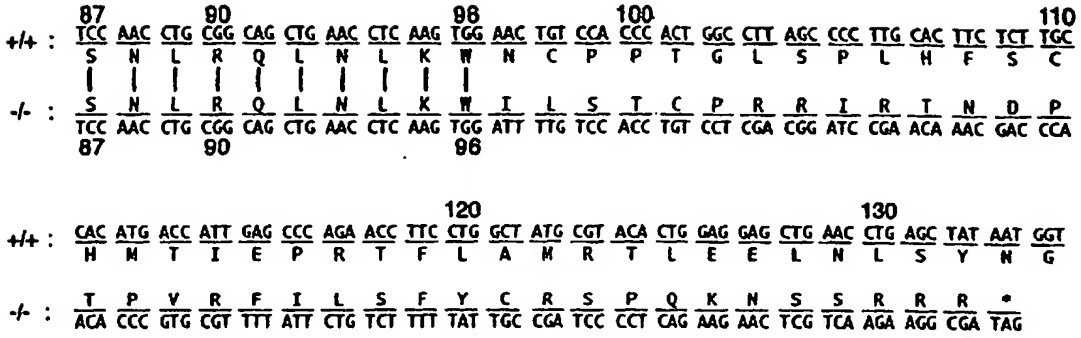

+/+ :
-/- :
87
90
96
100
110
120
130

FIG. 5

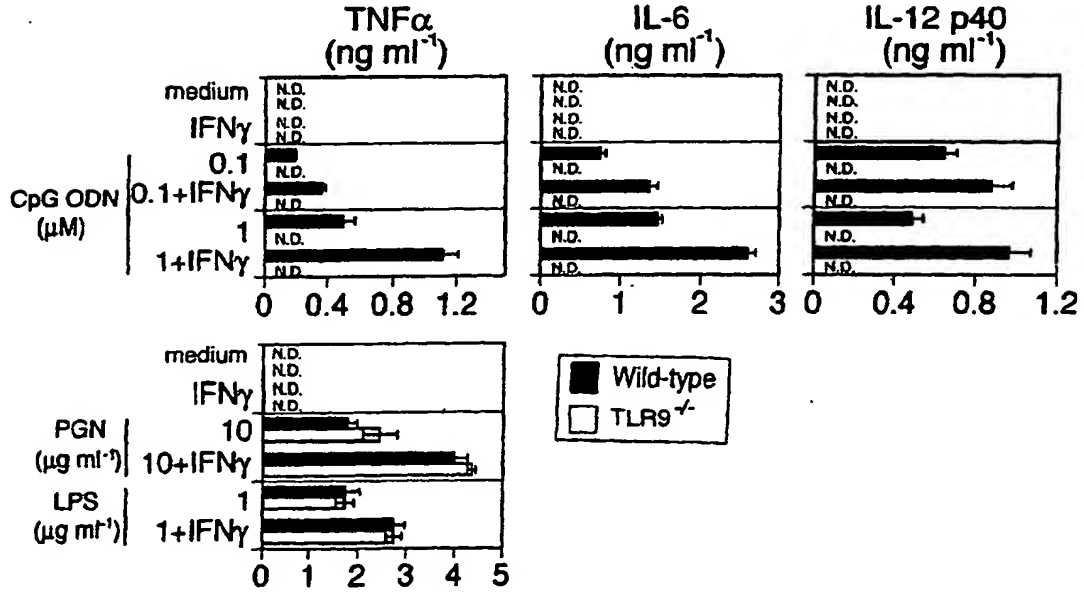
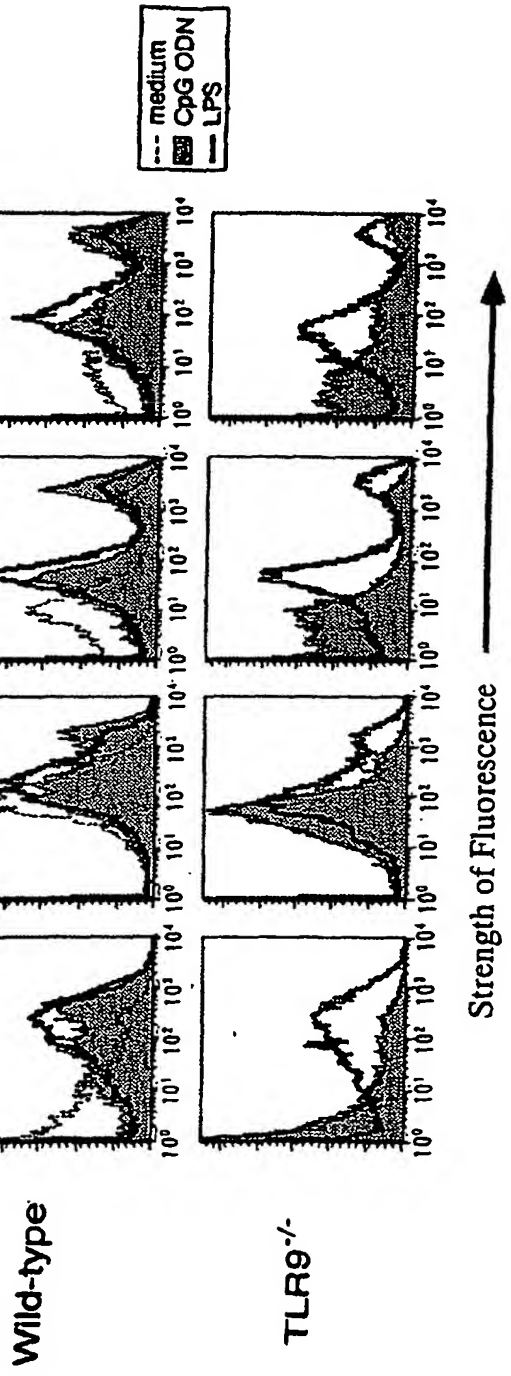

TNFα (ng ml-1)
IL-6 (ng ml-1)
IL-12 p40 (ng ml-1)
medium
IFNγ
CpG ODN (μM)
0.1
0.1+IFNγ
1
1+IFNγ
PGN (μg ml-1)
10
10+IFNγ
LPS (μg ml-1)
1
1+IFNγ
N.D.
Wild-type
TLR9-/-

FIG. 8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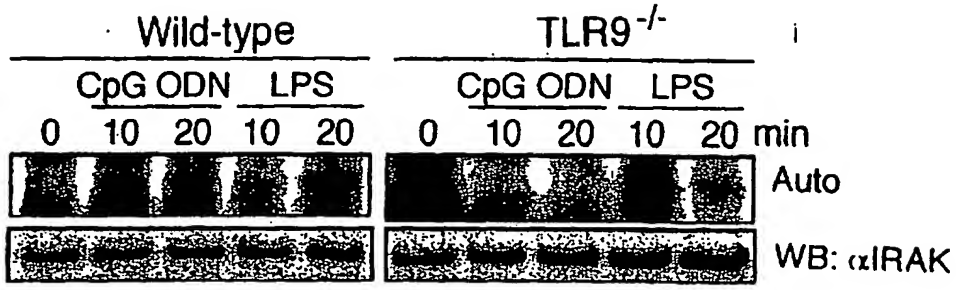
CpG ODN
LPS
MHC class II
CD86
CD80
CD40
Wild-type
TLR9-/-
Strength of Fluorescence

FIG. 11


Wild-type
TLR9 -/-
CpG ODN
LPS
CpG ODN
LPS
0 10 20 10 20
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Auto
WB: αIRAK

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/04731

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KOPP E.B. et al. The Toll-receptor family and control of innate immunity. Curr. Opin. Immunol. 1999, Vol. 11, No. 1, pages 13-18	1-26,28,30
A	TAKEUCHI O. et al. TLR6: A novel member of an expanding Toll-like receptor family. Gene 1999, Vol. 231, pages 59-65	1-26,28,30
A	CHAUDHARY P. M. et al. Cloning and characterization of Two Toll/Interleukin-1 Receptor-Like Genes TIL3 and TIL4:Evidence for a Multi-Gene Receptor Family in Humans. Blood 1998, Vol. 91, No.11, pages 4020-4027	1-26,28,30
A	ROCK F. L. et al. A family of human receptors structurally related to Drosophila Toll. Proc. Natl. Acad. Sci. USA 1998, Vol.95, pages 588-593	1-26,28,30
A	FEARON D.T. et al. Seeking wisdom in innate immunity. Nature 1998, Vol. 388, pages 323-324, 94-397	1-26,28,30
A	WO 99/51259 A2 (UNIV.IOWA RES.FOUND.), 14 October, 1999 (14.10.99), & AU 9934678 A & EP 1067956 A2 & US 6218371 B1	1-26,28,30
A	Krieg A.M. The role of CpG motifs in innate immunity. Curr. Opin. Immunol. February 2000, Vol. 12, No.1, pages 35-43	1-26,28,30
A	TAKEUCHI O. et al. Celluler responses to bacterial cell wall components are mediated through MyD88-dependent signaling cascades. Int. Immunol. January 2000, Vol.12, No.1, pp.113-117	1-26,28,30

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/04731

Continuation of Box No.I-2 of continuation of first sheet(1)

The agonist or antagonist as set forth in claim 27 and the medicinal composition as set forth in claim 29 are specified by the screening methods described in claims 23 to 26. Thus, any agonists or antagonists and medicinal compositions obtained by these screening methods are involved in the scopes thereof.

However, the description discloses no particular agonist, antagonist or medicinal composition obtained by these screening methods. Namely, claims 27 and 29 are neither supported nor disclosed by the description. Even though the common technical knowledge at the point of the application is taken into consideration, it is extremely unclear what particular compounds are involved in the scopes thereof and what are not. Thus, these claims are described in an extremely unclear manner.

Such being the case, no meaningful search can be practiced on the inventions as set forth in the above claims.

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